Check List the journal of biodiversity data

NOTES ON GEOGRAPHIC DISTRIBUTION

Check List 14 (6): 1077–1082
https://doi.org/10.15560/14.6.1077

PENSOFT

Discovery of wild populations of *Betta smaragdina* Ladiges, 1972 (Teleostei, Osphronemidae) in a western province of Thailand

Prachaya Pammanasut[1], Bhinyo Panijpan[2], Saengchan Senapin[2, 3], Pintip Ruenwongsa[2], Namkang Sriwattanarothai[4], Parames Laosinchai[4], Kornsunee Phiwsaiya[2, 3]

1 Wild Betta Conservation and Development Association, 16 Baan Kingpetch soi 10, Petchburi Road, Ratchathewi, Bangkok 10400, Thailand. **2** Center of Excellence for Shrimp Molecular Biology and Biotechnology, Mahidol University, 272 Rama 6 Road, Phayathai, Bangkok 10400, Thailand. **3** National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 111 Thailand Science Park, Phahonyothin Road, Khlong Nueng, Khlong Luang, Pathum Thani 12120, Thailand. **4** Institute for Innovative Learning, Mahidol University, 999 Phutthamonthon 4 Road, Salaya, Nakhon Pathom 73170, Thailand.
Corresponding author: Bhinyo Panijpan, bhinyop@gmail.com

Abstract

The bubble-nesting *Betta smaragdina* Ladiges, 1972 can be distinguished from the 4 other species of bubble-nesting *Betta* Bleeker, 1850 by being reportedly confined to Northeastern Thailand. We found large populations of fish in Western Thailand that closely resemble those from the Northeastern Region. The new populations inhabit a variety of places encompassing lakes, marshes, and streams. Morphological studies of *Betta* fish from the 2 well-separated regions, together with DNA analyses, show that the western and the northeastern populations are the same species.

Key words

DNA sequences; morphology; Kanchanaburi.

Academic editor: Henrique Lazzarotto de Almeida | Received 8 July 2018 | Accepted 2 November 2018 | Published 16 November 2018

Citation: Pammanasut P, Panijpan B, Senapin S, Ruenwongsa P, Sriwattanarothai N, Laosinchai P, Phiwsaiya K (2018) Discovery of wild populations of *Betta smaragdina* Ladiges, 1972 (Teleostei, Osphronemidae) in a western province of Thailand. Check List 14 (6): 1077–1082. https://doi.org/10.15560/14.6.1077

Introduction

Freshwater fishes of the genus *Betta* Bleeker, 1850 are found in most of Southeast Asia countries, except in the Philippines and perhaps Myanmar. In Thailand, there are 5 species of wild bubble-nest building *Betta* (Panijpan et al. 2017). These are *B. splendens* Regan, 1909 found in the Northern, Central, Upper Southern, Eastern, and Western regions including Kanchanaburi Province; *B. imbellis*, Ladiges 1975 in the Lower Southern; *B. siamorientalis* Kowasupat, Panijpan, Ruenwongsa & Jeenthong, 2012 in the Eastern; *B. mahachaiensis* Kowasupat, Panijpan, Ruenwongsa & Sriwattanarothai, 2012 in a small pocket west of Bangkok near the Gulf of Thailand; and *B. smaragdina* Ladiges, 1972 with populations confined to only 20 provinces in the northeastern Thailand (Fig. 1). Most of these *Betta* fishes have habitats confined to specific regions (Lertpanich and Aranyavalai 2007, Linke 2014, Goldstein 2015, Panijpan et al. 2017). One exception is *B. splendens*, which shares some habitats with other regional *Betta* species. These 5 species can be distinguished from one another morphologically, especially when the fully-grown males are in full aggressive display. Among these Thailand *Betta* fishes, cryptic species within *B. smaragdina* (Kowasupat et al. 2014) are the only ones with tens of bluish-green iridescent plates

Copyright *Pammanasut et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

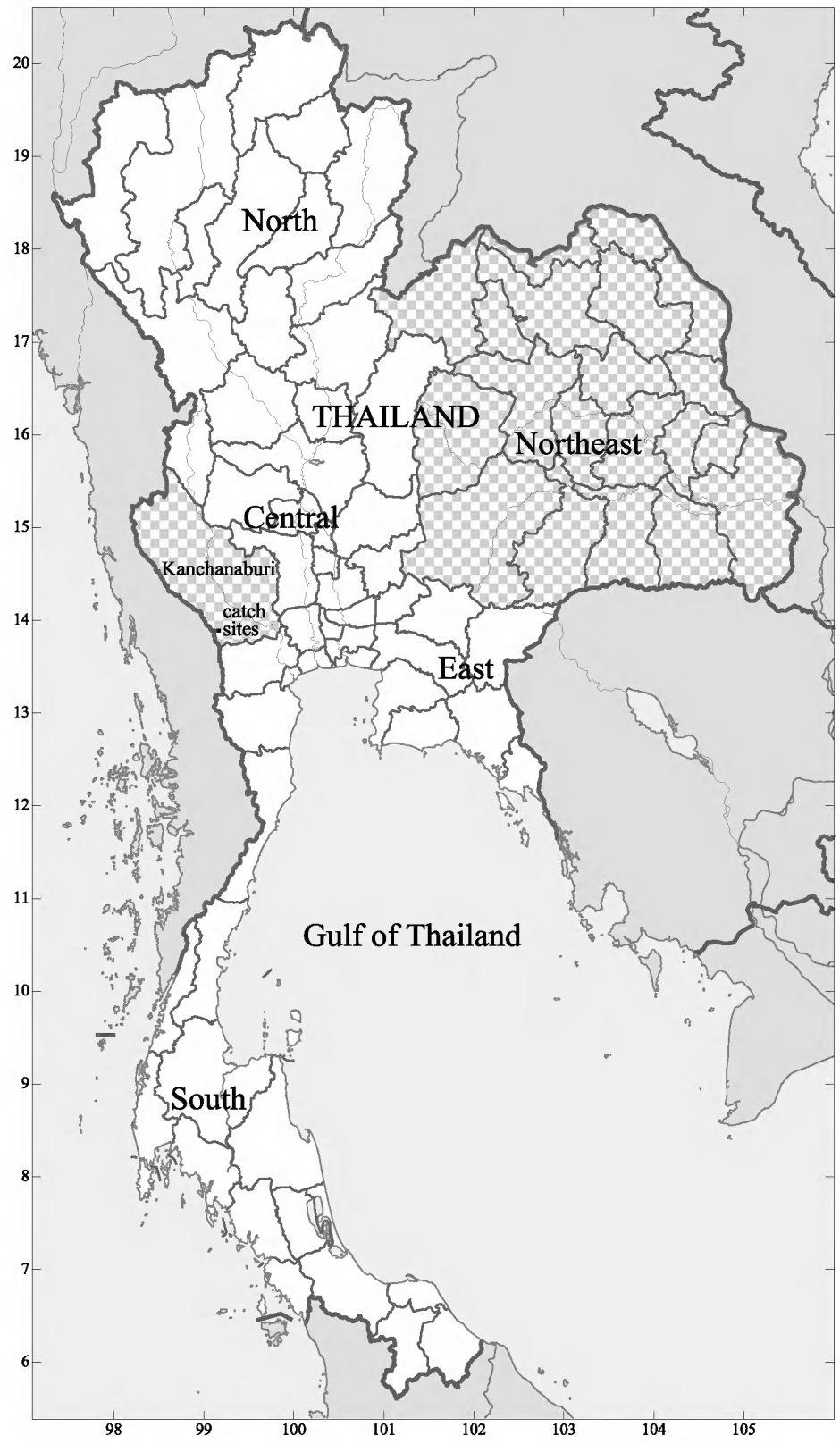

Figure 1. Map showing locations of collection sites of *Betta smaragdina* in the Northeastern Region and Kanchanaburi Province, Thailand.

on the head region covering the opercles down to the lower part of the mouth region. These plates are usually well separated and can be very small. So far, wild *Betta* fish with this unique character have never been reported elsewhere outside the Northeastern Region (Linke 2014, Goldstein 2015). Here, we report western populations of *Betta* from 5 collection sites (covering ca 6 km^2) in Kanchanaburi Province of Thailand, and use morphological and DNA evidence to confirm that fish from western and northeastern Thailand belong to the same species.

Methods

Sample collection. The collection sites (Table 1) are relatively inaccessible areas near the Thailand–Myanmar border. All sites, except for K07, are located in areas less than 5 km away from the border (Fig. 2). We caught the fish using hand-held netted tools, transported them in plastic bags and put them in glass jars (10 cm × 10 cm × 20 cm) individually in the laboratory for observation and caring. Dead specimens were deposited at the Natural History Museum, Thailand. Photographs of the 5 collection sites are shown in Figure 3A–E. We observed the fish in waterbodies several kilometers downstream from the collections sites but fish density was lower than in the collection sites.

Observation of morphological characteristics. We identified fully grown fish following Tan and Ng's (2005) taxonomic key, which was modified from Witte and Schmidt (1992). We recorded body color patterns, iridescence of body scales, facial iridescence plates, fin shape, fin size, fin rays, and fin color while they fully display their aggressive behavior (Fig. 4).

Molecular analysis. We cut a small part of the caudal fin of each specimen and put it into 95% ethanol for subsequent DNA analyses of the mitochondrial COI (cytochrome *c* oxidase subunit 1, and the nuclear RAG1 (recombination activating gene 1) and ITS1 (internal transcribed spacer 1) genes. Primers [VF2_t1 (Ivanova et al. 2007), FishF2_t1 (Ivanova et al. 2007), FishR_t1 (Ward et al. 2005) and FR1d_t1 (Ivanova et al. 2007)] and conditions for COI were employed following Ivanova et al. (2007). The nuclear RAG1 was amplified and sequenced with primers RAG1-2510F (Li and Ortí 2007) and RAG1-4090R (López et al. 2004) while ITS1 was amplified and sequenced with primers Betta_ITS1_F1 (Kowasupat et al. 2014) and Betta_ITS1_R1 (Kowasupat et al. 2014). These DNA sequences were deposited at GenBank (http://www.ncbi.nlm.nih.gov) (Table 2). We assembled and aligned the DNA sequences using Geneious version 5.6.7 (Kearse et al. 2012), and ana-

Table 1. New records of wild Western Thailand *Betta smaragdina* collected in this study. THNHM: Thailand Natural History Museum.

Record no.	Site	Latitude (N)	Longitude (E)	Alt. (m)	Locality	Life stage	Coll. date (No. of spec./ expedition)	Collector	Voucher specimen
1	K01	13° 53.64′	99° 8.41′	140	Lake in Kanchanaburi Province, Thailand	Adult	31 Aug 2017 (10) 18 Jan 2018 (10)	Prachaya Pammanasut	THNHM-F-0015701
2	K02	13° 53.59′	99° 8.72′	140	Swamp marshes with various vegetation in Kanchanaburi Province, Thailand	Adult	31 Aug 2017 (10) 18 Jan 2018 (10)	Prachaya Pammanasut	THNHM-F-0015702 THNHM-F-0015703
3	K03	13° 53.35′	99° 8.82′	130	Shallow stream (upwelling water) in Kanchanaburi Province, Thailand	Adult	31 Aug 2017 (10) 18 Jan 2018 (10)	Prachaya Pammanasut	THNHM-F-0015704
4	K04	13° 52.97′	99° 8.45′	150	Swamp marshes in banana groves of Kanchanaburi Province, Thailand	Adult	31 Aug 2017 (10) 18 Jan 2018 (10)	Prachaya Pammanasut	THNHM-F-0015705
5	K07	13° 53.14′	99° 10.12′	120	Swamp marsh along road in Kanchanaburi Province, Thailand	Adult	31 Aug 2017 (10) 18 Jan 2018 (10)	Prachaya Pammanasut	THNHM-F-0015709

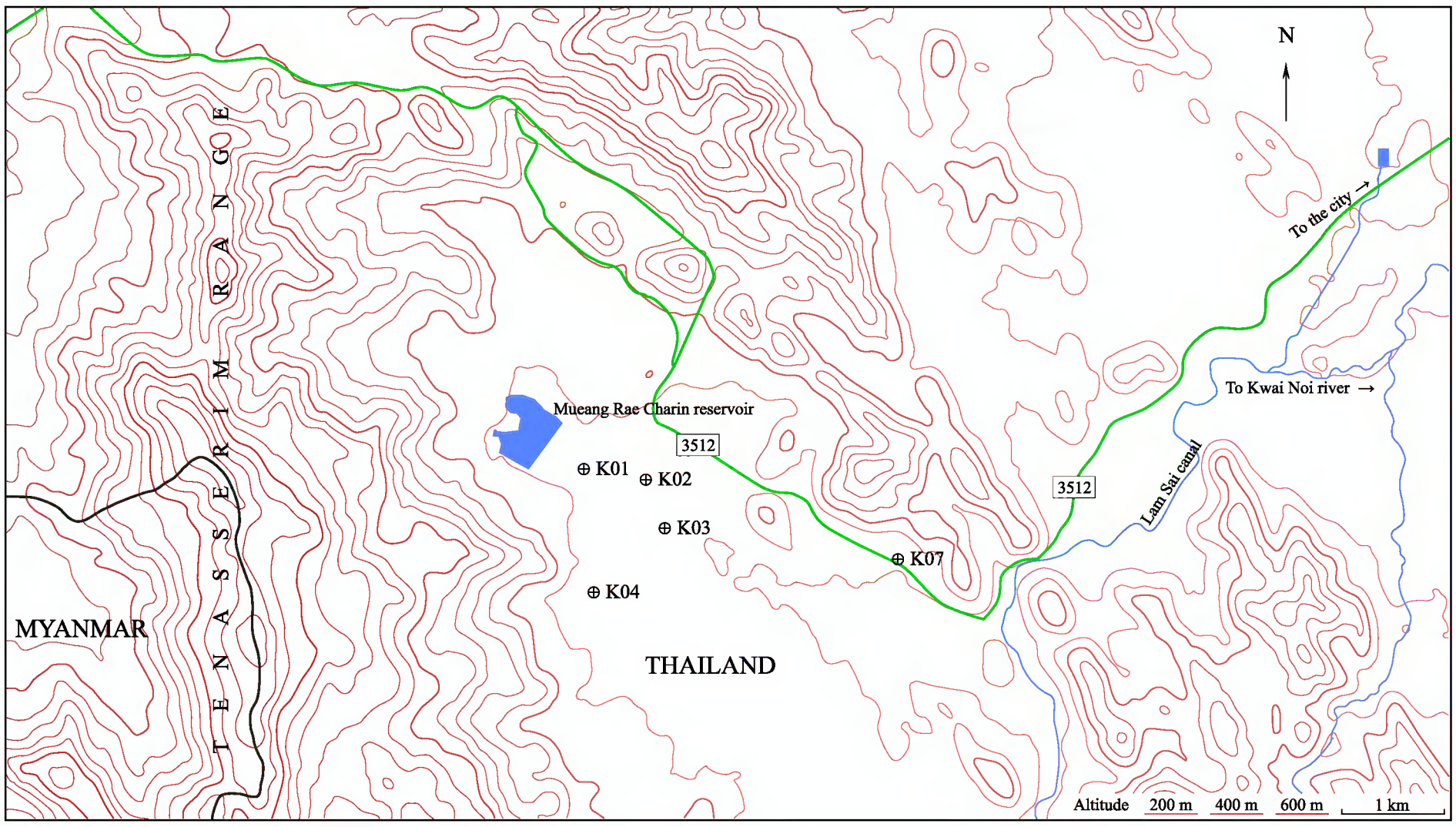

Figure 2. Magnified contour map of Kanchanaburi Province showing 5 collection sites. Adapted from the map originated by NordNordWest and modified by Paul_012. The map is available under the Creative Commons Attribution-Share Alike 3.0 from https://en.wikipedia.org/wiki/ File:Thailand_provinces_en.svg (2 August 2017).

lysed them using MrBayes version 3.2.6 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003, Ronquist et al. 2012) after partitioning them according to their locations (mitochondrion versus nucleus) and codon positions. Based on the parameters sampled from a preliminary posterior distribution, we adjusted the model used in the Bayesian inference until the marginal likelihoods could not be significantly improved.

Results

New records. Table 1.

Identification. We identified the new fish by comparing it with *B. smaragdina* from all parts of northeastern Thailand. Figure 4 shows the comparative photographs of *B. smaragdina* from Western and Northeastern regions. The western *Betta* fish are practically identical with *B. sma-*

Table 2. Locations of collection sites of *Betta* fish of Thailand used in the DNA analysis. ACNO: GenBank accession number. KP: Kanchanaburi Province. *N*: number of examined specimens.

Species	*N*	Site/Region	Locality	ACNO			Reference
				COI	ITS1	RAG1	
Betta smaragdina KP	1	K01	Lake in KP	MH065735	MH065743	MH065739	This study
	1	K03	Shallow stream (upwelling water) in KP	MH065736	MH065744	MH065740	This study
	1	K04	Swamp marshes in banana groves of KP	MH065737	MH065745	MH065741	This study
	1	K07	Swamp marsh along road in KP	MH065738	MH065746	MH065742	This study
Betta smaragdina	1	Upper Northeast	Lake in Nong Khai Province	JQ818762	JQ818588	MH425149	Kowasupat et al. 2014
	1	Lower Northeast	Marsh along road in Nakhon Ratchasima Province	KF278890	KF381311	MH425150	Kowasupat et al. 2014
Betta sp. (cf. *smaragdina*) 1	2	Upper Northeast	Margins of a big lake in Bueng Kan Province	JQ818752, KF278838	JQ818585–6	MH425146–7	Kowasupat et al. 2014
	1	Upper Northeast	Margins of a big lake in Bueng Kan Province	KF278839	JQ818587	MH425148	Kowasupat et al. 2014
Betta mahachaiensis	1	Central	Nipa palm forest near a tributary in Samut Sakhon Province	JQ818677	JQ818568	MH425143	Kowasupat et al. 2012a
	1	Central	Nipa palm forest in suburb Samut Sakhon Province	JQ818683	JQ818572	MH425144	Kowasupat et al. 2012a
	1	Central	Nipa palm forest in Samut Sakhon Province near border of Bangkok	JQ818693	JQ818574	MH425145	Kowasupat et al. 2012a
Betta splendens	1	East	Marsh along road in Chanthaburi Province	JQ818727	JQ818639	MH425142	Kowasupat et al. 2012a
	1	South	Paddy field near mountains in Prachuap Khiri Khan Province	KF278853	KF381315	MH425154	Kowasupat et al. 2014
Betta siamorientalis	1	East	Swamp marsh near river in Chachoengsao Province	JQ818714	JQ818629	MH425141	Kowasupat et al. 2012b
Betta imbellis	1	South	Marsh in suburb Songkhla Province	MH065734	JQ818594	MH425151	Kowasupat et al. 2012a
	1	South	Swamp marsh near lake within national park in Songkhla Province	JQ818781	JQ818605	MH425152	Kowasupat et al. 2012a
	1	South	Swamp marsh near canal in Nakhon Si Thammarat Province	JQ818783	JQ818607	MH425153	Kowasupat et al. 2012a

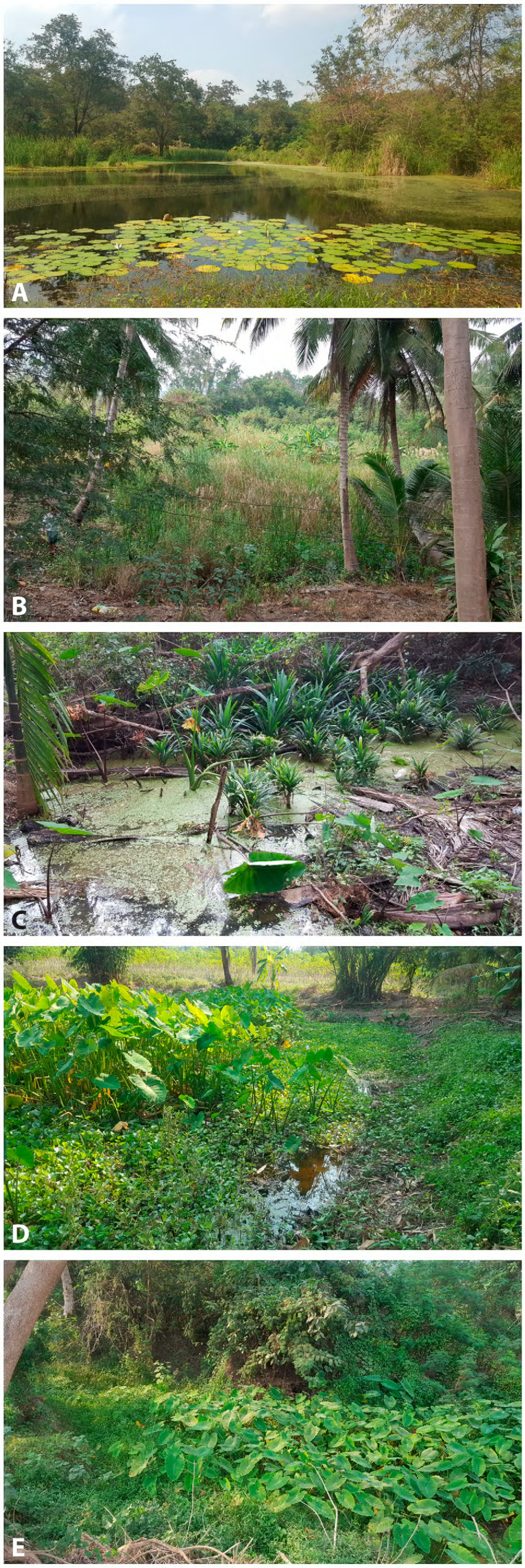

Figure 3. Photographs of various collection sites of *Betta smaragdina* in Kanchanaburi (K) Province. **A.** Site K01. **B.** Site K02. **C.** Site K03. **D.** Site K04. **E.** Site K07.

ragdina from the Upper Northeastern Region with only minor differences.

Visible shared features. Kanchanaburi *Betta* fish shares a distinct feature in the head part, anterior to the gill openings, with the type-locality *B. smaragdina* from the northeastern Thailand. Both groups of fish have more than 30 well-separated bluish-green iridescent plates located in the ventral area of the head. The second shared feature is the caudal fin, having about 12 rays originating from the peduncle and splitting into 2 toward the posterior end of the fin. The third shared feature is 8 clear rows of very bright iridescent scales running laterally on both sides of the body.

Visible minor differences. Based on 5 male fish from each location, fully grown *B. smaragdina* (3.8–4.2 cm standard length) and Kanchanaburi fish (3.5–3.9 cm standard length) showed slight differences in the ratios of pelvic fins to body length, 0.43 + 0.03 for *B. smaragdina* and 0.32 + 0.03 for Kanchanaburi fish. To the naked eye, the body of *B. smaragdina* appeared more slender than Kanchanaburi fish, however, ratios of the broadest part of the body/standard length are 0.25 + 0.01 for *B. smaragdina* and 0.27 + 0.01 for Kanchanaburi fish.

DNA analysis. The DNA sequences from the mitochondrion and nucleus show the new fish to be practically identical to *B. smaragdina* from the type locality. COI, RAG1, and ITS1 sequences of the Kanchanaburi bettas, when compared to those from GenBank and our unpublished RAG1 data from all varieties of *B. smaragdina* (Table 2), show clearly that these Kanchanaburi fish belong to the same clade as *B. smaragdina* from the northern part of the northeastern Thailand. In particular, COI sequences of Kanchanaburi fish are practically identical (99.7–99.8% sequence identity) to that of a typical specimen of *B. smaragdina* from the Upper Northeastern Region while their RAG1 sequences present 99.4–99.7% identity. In addition, all informative sites in the RAG1 sequences from both western and northeastern *B. smaragdina* fish were indistinguishable. As for ITS1 sequences, the differences are 2–3 indels whose lengths are 1, 2 and 4 base pairs. The non-indel sites present 99.8–100% sequence identity. Figure 5 depicts the phylogenetic tree reconstructed from the combined DNA sequences (COI, RAG1, and ITS1). It is interesting that *B. splendens*, *B. mahachaiensis*, and *B. siamorientalis* have no cryptic species—populations phylogenetically separable by distinct DNA sequences. However, *B. smaragdina* has at least 4 cryptic species; only 2 of which were analyzed (Fig. 5).

Discussion

As mentioned earlier, there are other locations in Kanchanaburi Province where only *B. splendens* and no other *Betta* spp. are found. *Betta smaragdina* has so far been assigned solely as a northeastern Thailand fighting fish since its first description by Ladiges (1972). More recently, in the Western Region, which is well-separated

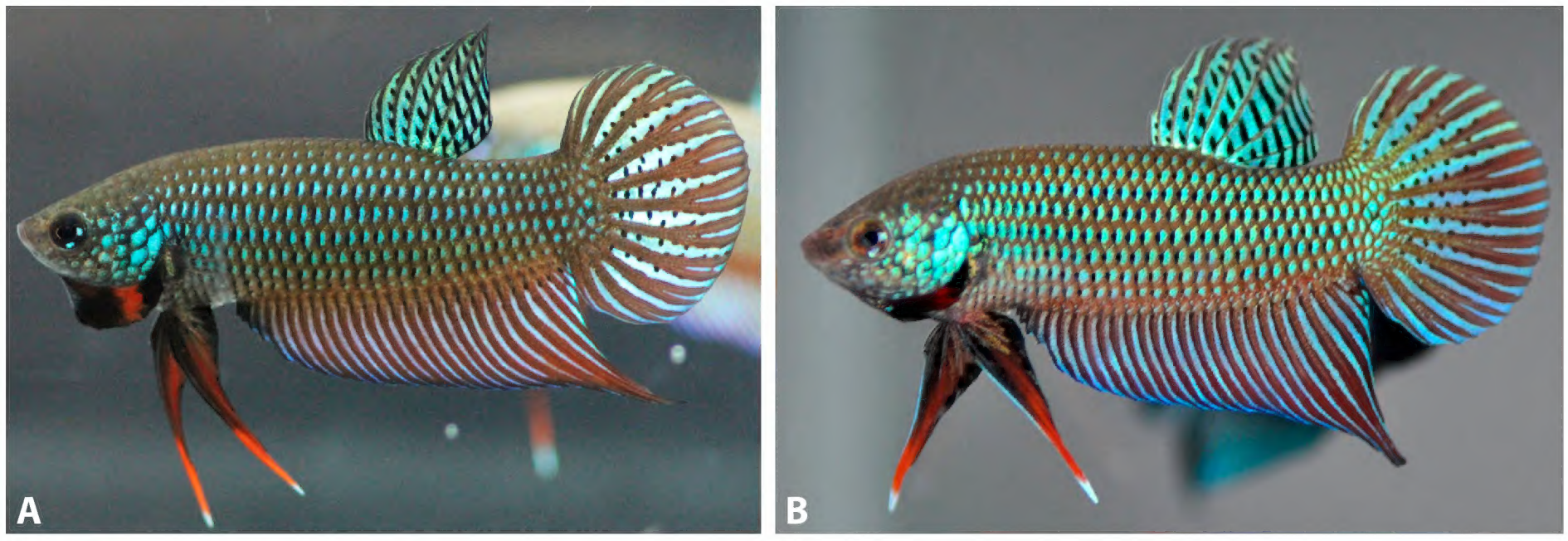

Figure 4. Photographs of *Betta smaragdina* , in full aggressive display, from: **A.** Northeastern Thailand. **B.** Kanchanaburi Province.

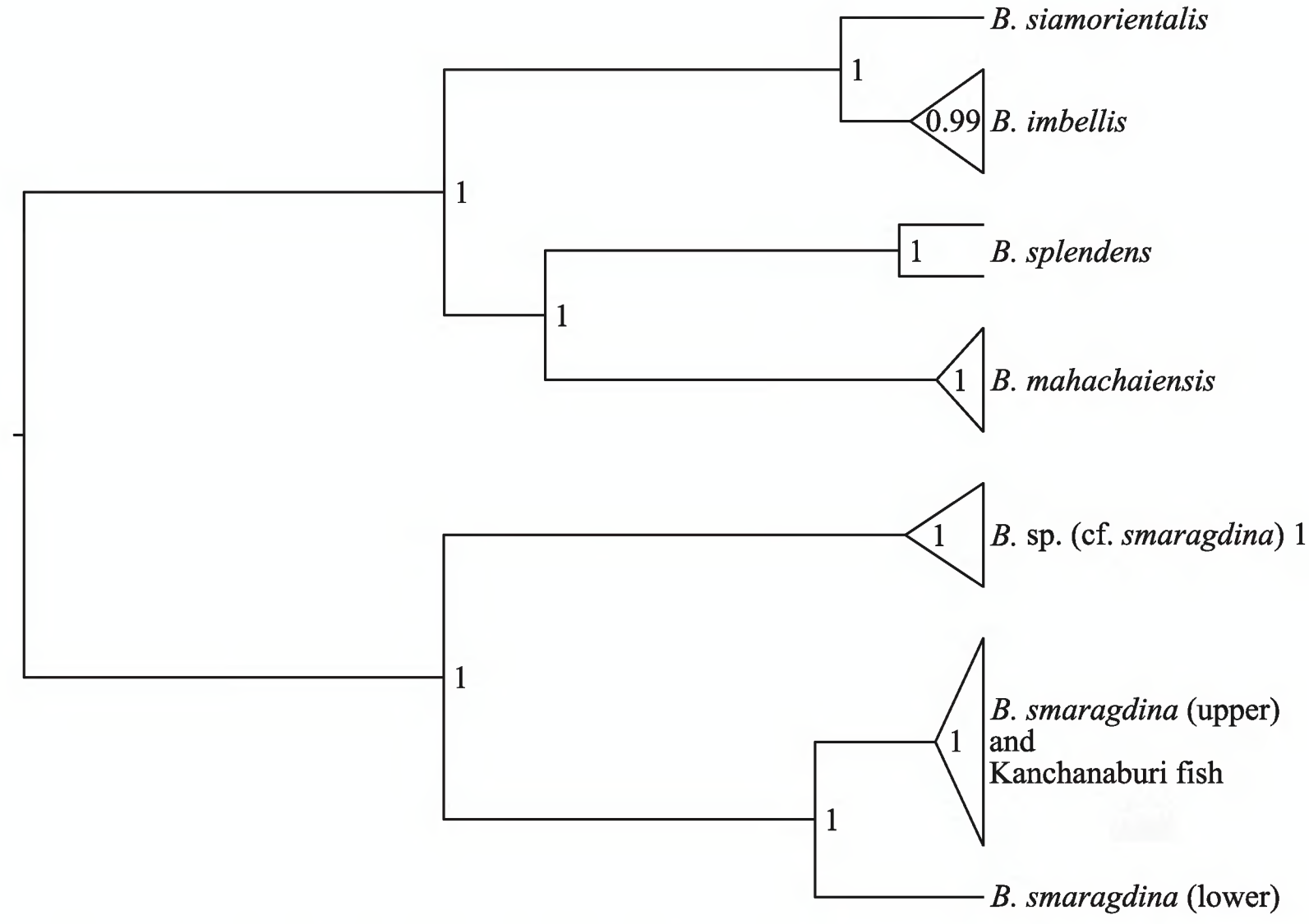

Figure 5. Phylogenetic tree reconstructed from analysis the combined DNA sequences (COI, RAG1, and ITS1). The number on each node represents the posterior probability supporting that clade. Upper and lower indicate northern and southern parts of the Thailand Northeast.

from the Northeast Region, *B. smaragdina* has been found in abundance over a relatively wide area covering various types of waterbodies, such as lakes, ponds, and streams. However, fish in these 2 regions live in water of different transparency. In the west, all collection sites have clear water that mostly originates from high elevations in mountains flowing down to lower altitudes (Fig. 1), whereas in the northeast, on the plateaus, turbid water is accumulated from rain and upwelling underground water. The morphological differences, for example, the pelvic fins and anal fin, are extremely minor between the fish from the 2 regions. In addition, their DNA sequences (COI, RAG1, and ITS1) are practically identical. If these fish had been translocated from one region to the other, the time of the separation of the fish from the 2 regions must not have been long enough for the fish to change its DNA sequences, but some minor morphological adaptations could have taken place.

Analyses of COI, RAG1, and ITS1 sequences of fish used for genetic identification of species (Hebert et al. 2003), show that they differ from those of *B. mahachaiensis* and any other Thailand bubble-nesting *Betta* species. Some features in western *Betta*, especially scale iridescence and body shape, may remind some people of *B. mahachaiensis*. However, as there is no indication of contamination by *B. mahachaiensis* DNA in our western fish, it is safe to say these morphological similarities between western *B. smaragdina* and *B. mahachaiensis* are homoplastic.

From our observation, western fish have thrived so well to populate these collection sites and beyond, where there are no other bubble-nesting species. Thus one could

only speculate on how and when large populations of the same species have come to occupy different habitats in the Northeast and the West regions, which are separated by great distances. One proposal, that the fish of both regions emerged more or less simultaneously in the past, is not tenable because their DNA sequences are practically identical. Such coincidence is implausible. The northeastern *B. smaragdina* populations harbor at least 4 cryptic species, indicating the evolutionary time of over 5 million years. But there is only 1 of the 4 species in the Northeastern Region that is identical with the western Kanchanaburi fish.

The present sparse populations of Karen tribes, probably recent migrants from Myanmar, living near the sites do not know these tiny fish, which are considered by them to be too small to eat, nor do they have the game of fish fighting. These people are not the ones who brought the northeastern fish to the west. Moreover, these sites have been hidden from Thai and foreign aquarists because modern road transportation has been made possible only in the last 10 years.

Acknowledgements

We thank Mr Chanchai Sunan-kingpeth, Mr Chaichana Kaewkheao, Mr Phrommart Sieangpream, and Mr Phichet Santtanavanich for their assistance in the expeditions, especially, PS for photos of Kanchanaburi fish. We also thank Prof. Tim Flegel for reading the manuscript and his suggestions.

References

Goldstein RJ (2015) The *Betta* Handbook. Barron's Educational Series, New York, 176 pp.

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society of London, Series B 270: 313–321. https://doi.org/10.1098/rspb.2002.2218

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN (2007) Universal primer cocktails for fish DNA barcoding. Molecular Ecology Notes 7: 544–548. https://doi.org/10.1111/j.1471-8286.2007.01748.x

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kowasupat C, Panijpan B, Ruenwongsa P, Sriwattanarothai N (2012a) *Betta mahachaiensis*, a new species of bubble-nesting fighting fish (Teleostei: Osphronemidae) from Samut Sakhon Province, Thailand. Zootaxa 3522: 49–60.

Kowasupat C, Panijpan B, Ruenwongsa P, Jeenthong T (2012b) *Betta siamorientalis*, a new species of bubble-nest building fighting fish (Teleostei: Osphronemidae) from eastern Thailand. Vertebrate Zoology 62: 387–397.

Kowasupat C, Panijpan B, Laosinchai P, Ruenwongsa P, Phongdara A, Wanna W, Senapin S, Phiwsaiya K (2014) Biodiversity of the *Betta smaragdina* (Teleostei: Perciformes) in the northeast region of Thailand as determined by mitochondrial COI and nuclear ITS1 gene sequences. Meta Gene 2: 83–95. https://doi.org/10.1016/j.mgene.2013.12.004

Ladiges W (1972) *Betta smaragdina* nov. spec. Aquarien und Terrarien Zeitschrift 25: 190–191.

Lertpanich K, Aranyavalai V (2007) Species diversity, distribution and habitat characteristic of wild bubble nesting betta (*Betta* spp.) in Thailand. KMITL Science Journal 7: 37–42.

Li C, Ortí G (2007) Molecular phylogeny of Clupeiformes (Actinopterygii) inferred from nuclear and mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 44: 386–398. https://doi.org/10.1016/j.ympev.2006.10.030

Linke H (2014) Labyrinth Fish World. Fish Magazine Taiwan, Taiwan, 577 pp.

López JA, Chen W-J, Ortí G (2004) Esociform phylogeny. Copeia 2004 (3): 449–464. https://doi.org/10.1643/CG-03-087R1

Panijpan B, Sriwattanarothai N, Kowasupat C, Ruenwongsa P, Jeenthong T, Phumchoosri A (2017) Biodiversity of bubble-nest building and mouth-brooding fighting fish species of the genus *Betta* in Southeast Asia. Thailand Natural History Museum Journal 11 (1): 1–21.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Tan HH, Ng PKL (2005) The fighting fishes (Teleostei: Osphronemidae: genus *Betta*) of Singapore, Malaysia and Brunei. The Raffles Bulletin of Zoology Supplement 13: 43–99.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B 360: 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Witte K, Schmidt J (1992) *Betta brownorum,* a new species of anabantoids (Teleostei; Belontiidae) from northwestern Borneo, with a key to the genus. Ichthyological Exploration of Freshwaters 2: 305–330.